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Effect of calcium, sodium and potassium on adrenal tyrosine hydroxylase activity *in vitro*

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THE REGULATION of synthesis of catecholamines (CA) is currently thought to be mainly through the inhibitory effect of CA on the rate limiting step, i.e. tyrosine hydroxylase (TH).[1] The regulatory function is ascribed to a specific "pool" of CA which presumably interacts with this enzyme, although such a pool has not been clearly defined.[2] However, inhibition of TH by CA can be demonstrated *in vitro*.[3]

A different type of experiment has shown a mechanism regulating CA synthesis *in vivo* through increased sympathetic stimulation.[4,5] This has been coined as "trans-synaptic induction" and involves increased synthesis of TH by prolonged presynaptic stimulation.[5] In addition to the gradual increase of TH following presynaptic stimulation, which is due to synthesis of the enzyme, an immediate increase in conversion of tyrosine to noradrenaline (NA) has been demonstrated *in vivo* even with a short stimulation, when new synthesis of TH cannot account for the enhanced NA synthesis.[6] This immediate increase in NA synthesis may be ascribed to depletion of the specific NA pool mentioned above.[2] However, several other phenonena accompany nerve stimulation to the adrenal medulla.

For example changes in permeability for Na^+ and Ca^{2+}, which may cause influx and increased intracellular concentrations of Na^+ and Ca^{2+} in chromaffin cells.[7] TH assays *in vitro* are frequently carried out in a medium consisting mainly of Na^+[8] whereas the enzyme *in vivo* is intracellular and, therefore, in a predominantly K^+ medium. Therefore, we studied the effect of $Na,^+$ K^+ and Ca^{2+} on TH activity *in vitro*.

Tyrosine hydroxylase was prepared from bovine adrenal. The adrenal medulla was homogenized in 10 vol. of 0·32 M sucrose. The homogenate was subjected to centrifugation at 1000 *g* for 10 min and the supernatant was spun at 17,000 *g* for 60 min. TH activity was assayed in the supernatant of the last centrifugation.

TH activity was assayed according to the method of Nagatsu *et al.*[8] The incubation medium consisted of 100 μmoles of acetate buffer pH 6·0; 100 μmoles mercaptoethanol, 0·5 μmole ferrous-ammonium sulfate, 1 μmole pargyline, 1 μmole decarboxylase inhibitor (RO-4-4602/1 of Hoffman-La Roche), 2 μmoles of 2-amino-6,7-dimethyl-4-hydroxy-5,6,7,8 tetrahydropteridine hydrochloride, 0·2 ml of the enzyme. Final volume was 1·0 ml. 0·5 μc of 1-tyrosine-3,5-H^3 was added (Radiochemical Center, Amersham, specific activity 52 c/m-mole). Incubation was carried out at 37° in a shaking bath for 15 min. The reaction was stopped by adding 0·1 ml of 3 M trichloroacetic acid (TCA). In the blanks TCA was added before incubation. The incubation mixture was applied on a column of DOWEX 50W × 4 and the effluent plus washing with 1 ml of water were combined in a counting vial followed by addition of 10 ml of Bray's solution.[9] Packard Tri-Carb scintillation spectrometer was used for counting.

In experiments with an Na^+ medium the acetate buffer consisted of sodium acetate ($[Na^+] = 100$ mM), a K^+ medium consisted of potassium-acetate buffer ($[K^+] = 100$ mM) and the combined medium consisted of a potassium-acetate buffer plus NaCl ($[Na^+] = 30$ mM). Ca^{2+} was added as $CaCl_2$.

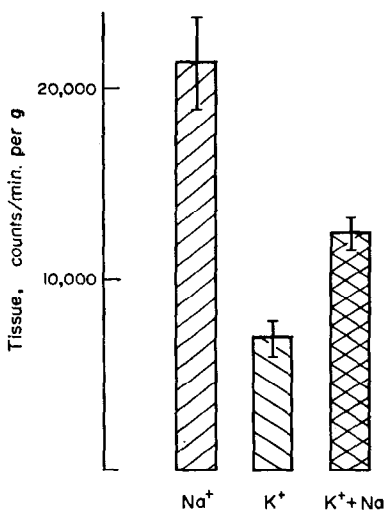

FIG. 1. Effect of sodium and potassium on adrenal tyrosine hydroxylase activity *in vitro*. Na^+, incubation medium with 100 mM Na^+ ($n = 6$); K^+, incubation medium with 100 mM K^+; and $K^+ + Na^+$, incubation medium with 100 mM K^+ and 30 mM Na^+ ($n = 10$). Vertical bars, S.E.M. The differences between K^+ and Na^+, between Na^+ and $K^+ + Na^+$ and between K^+ and $K^+ + Na^+$, $P < 0.01$.

Figure 1 shows that TH activity in a purely K^+ medium was significantly lower than in a purely Na^+ medium. When Na^+ was added to the K^+ medium (30 mM Na^+, 100 mM K^+) the TH activity increased significantly compared to the purely K^+ medium.

Table 1 shows the effect of Ca^{2+} on TH activity. A biphasic effect was observed. Adding 0·1 mM Ca^{2+} increased significantly TH activity compared to a Ca^{2+} free medium. However, further increase of $[Ca^{2+}]$ to 0·2 mM caused a significant inhibition of TH.

TABLE 1. EFFECT OF CALCIUM ON ADRENAL TYROSINE HYDROXYLASE ACTIVITY *in vitro*

Calcium concentration (mM)	Change in tyrosine hydroxylase activity*		t	p
	(cpm/g tissue)	(%)		
0·1 (n = 26)	+2822 ± 1043	+23.6	2·706	< 0·02
0·2 (n = 26)	−2236 ± 863	−18·7	2·591	< 0·02

* The change in activity compared to paired experiments where no Ca^{2+} was added to the incubation medium, $[K^+]$ = 100 mM, $[Na^+]$ = 30 mM. n, number of experiments.

These findings point to the possibility that, in addition to NA, changes in intracellular ionic composition may play some role in the regulation of CA synthesis through an effect on TH activity. Changes in ion fluxes which accompany nerve stimulation of the adrenal medulla are in the same direction as chosen in our experiments, i.e. increased influx of Ca^{2+} and Na^+[7]. The immediate increase of NA synthesis following stimulation could, therefore, be partly due to changes in intracellular ionic composition. It is also interesting to note that the range of $[Ca^{2+}]$ which increases TH activity is rather small and overlaps the range of intracellular $[Ca^{2+}]$. Further experiments on the mechanism of the effect of Ca^{2+}, Na^+ and K^+ on TH are now in progress.

While our experiments were in progress, Boadle-Biber *et al.*[10] have reported increased NA synthesis from tyrosine in isolated vas deferens exposed to high K^+ concentrations in the medium. This enhancement was evident only when Ca^{2+} was present in the medium. The findings were interpreted as due to release of NA by high $[K^+]$ in the medium with Ca^{++} being necessary for activation of NA release. However, depolarization of chromaffin cells by high $[K^+]$ is accompanied by increased Ca^{2+} influx.[7] It is noteworthy that at $[K^+]$ above 52 mM the increased NA synthesis was eliminated.[10] Our findings may point to increased intracellular Ca^{2+} as a direct mechanism affecting TH activity. With very high K^+ in the medium in the experiments of Boadle-Biber *et al.* it is also possible that Ca^{2+} influx into the cells increased Ca^{2+} concentration to a level producing inhibition (see Table 1).

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